

Repurposing of FDA Approved Drugs and Neuropep peptides as Anticancer Agents Against ErbB1 and ErbB2

Authors

Sunil Kumar Patnaik¹, Akey Krishna Swaroop¹, Mudavath Ravi Naik², Jubie Selvaraj¹, Moola Joghee Nanjan Chandrasekar^{1,3}, 

Affiliations

- 1 Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, The Nilgiris, Tamilnadu, India
- 2 Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, The Nilgiris, Tamilnadu, India
- 3, School of Life Sciences, JSS Academy of Higher Education & Research (Ooty Campus), Longwood, Mysuru Road, Ooty-643001, Tamilnadu, India

Key words

cytoscape, igemdock, hpepdock, datawarrior, *invitro* proliferation assay.

received 11.01.2023

accepted 30.01.2023

published online 17.04.2023

Bibliography

Drug Res 2023; 73: 341–348

DOI 10.1055/a-2030-4078

ISSN 2194-9379

© 2023, Thieme. All rights reserved.

Georg Thieme Verlag, Rüdigerstraße 14, 70469 Stuttgart, Germany

Correspondence

Dr. MJN Chandrasekar Professor
JSS College of Pharmacy “Rocklands” Post Box No.20
Udhagamandalam
643 001 Tamil Nadu State
INDIA
Tel.: 9486559781
ncsekar@jssuni.edu.in

ABSTRACT

ErbB1 and ErbB2 are the most important biological targets in cancer drug discovery and development of dual inhibitors for the cancer therapy. FDA approved drugs and Neuropep peptides were used to fit into the ATP binding site of the tyrosine kinases; ErbB1 and ErbB2 proteins. Cytoscape, iGEMDOCK, HPEPDOCK and DataWarrior softwares were used to study the role of these agents as anticancer drugs. Eleven FDA approved drugs and eleven Neuropep peptides showed the strongest 2D interactions and significant binding energy with the proteins. *In vitro* MTT anticancer assay revealed that, the test compounds, peptide YSFGL and doxorubicin showed significant IC₅₀ value (μM) of 26.417 ± 0.660 and 7.675 ± 0.278 respectively which are compared with the lapatinib standard IC₅₀ value (μM) of 2.380 ± 0.357 against A549 cells and IC₅₀ value (μM) of 39.047 ± 0.770 and 8.313 ± 0.435 respectively which are compared with the lapatinib standard IC₅₀ value (μM) of 3.026 ± 0.180 against MDA-MB-231 cells.

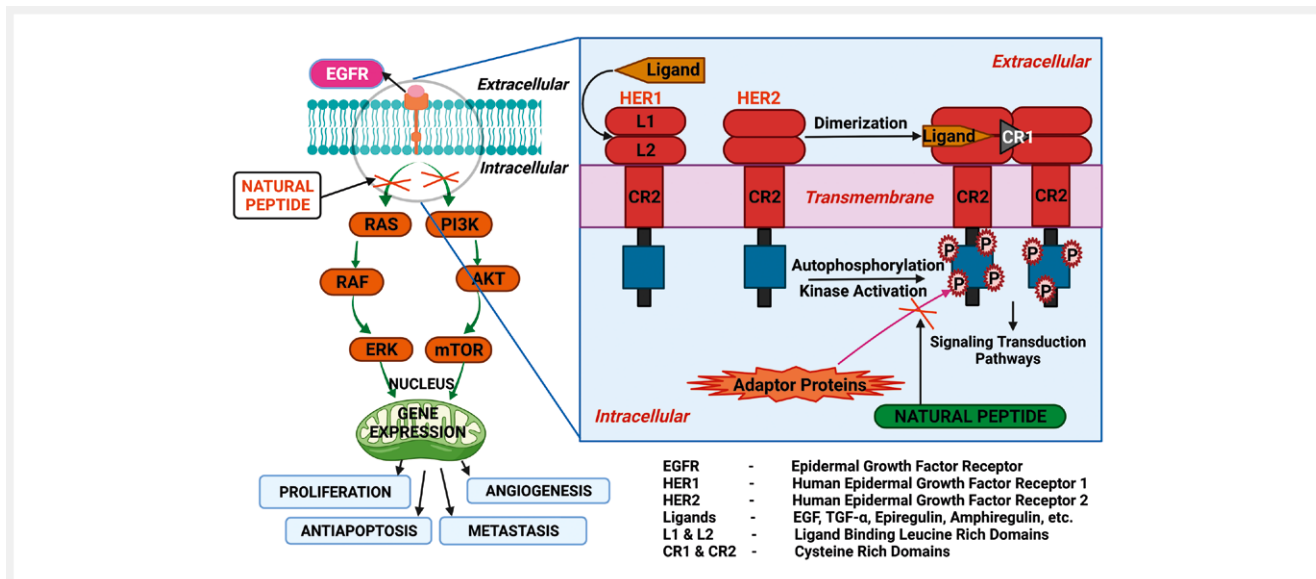
Introduction

Cancer is one of the most dangerous diseases that have a serious risk to the mankind's health and a great challenge to the contemporary medicine [1]. In general physiology of body system, apoptosis plays a key role in the regulation of the cell cycle progression by leading the faulty cells to a programmed cell death. Check points in the cell cycle identify the faulty cells [2]. Surgery, radiotherapy, chemotherapy, and immunotherapy are the therapeutic strategies used for cancer treatment. Chemotherapy is still the major approach to treat cancer but has a drawback of increased chances of resistance and recurrence of the disease [3]. Protein kinases play a major role in apoptosis, cell cycle progression, cell division, cytoskeletal rearrangement, cell differentiation and development, the immune response, nervous system dynamics, transcription,

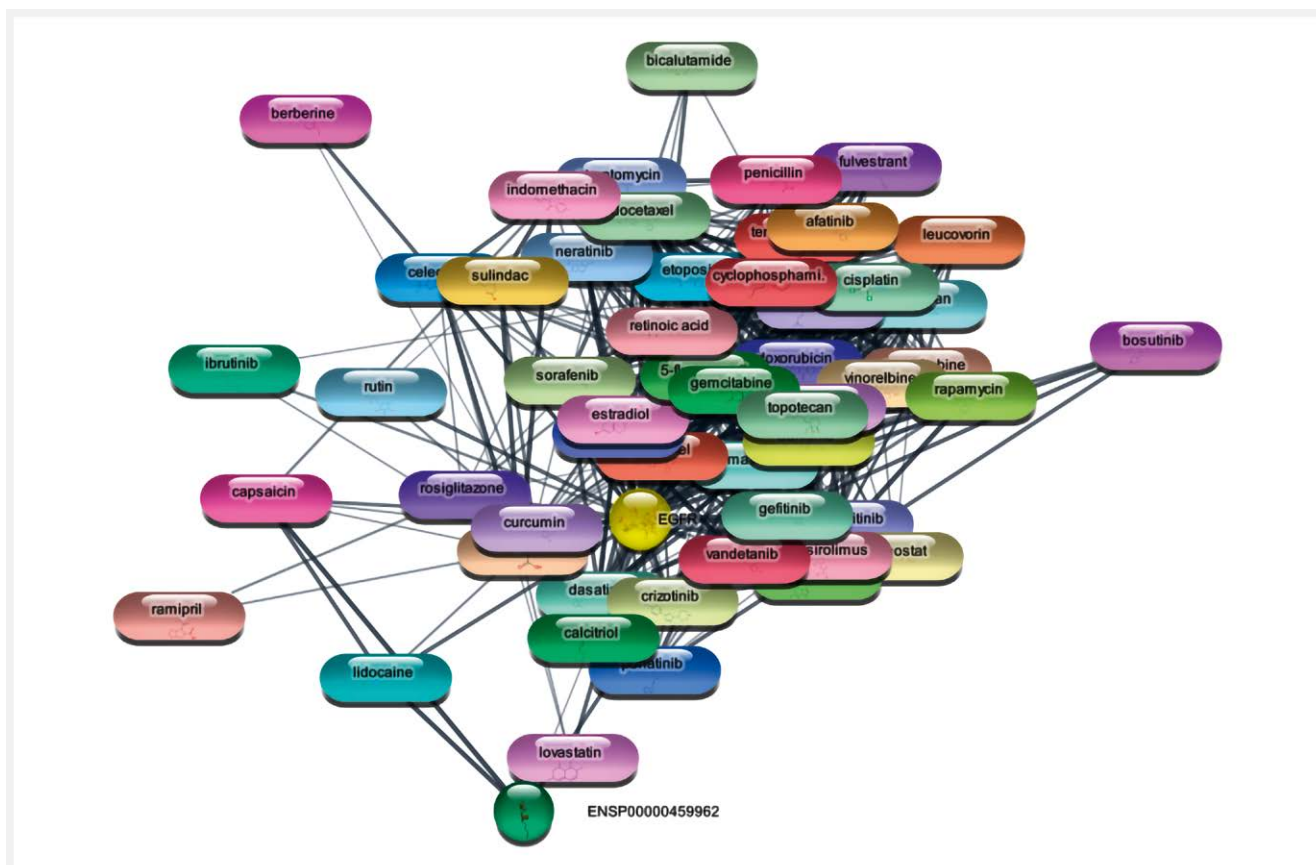
and translation. Dysregulation of these protein kinases activity play a prominent role in the cancer [4]. Anti-apoptosis, angiogenesis, tumor vascularization and tyrosine kinase activity play a major role in cell proliferation and metastases [1]. The ErbB receptor protein-kinases regulate apoptosis, cell cycle progression, development, metastases and invasion [5]. The ErbB/HER receptor tyrosine kinases are one of the most researched category of cell signaling families in cancer biology because of their roles in signal transduction and oncogenesis [6]. EGFR/ErbB1/HER1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4 are the four members belonging to the tyrosine kinase receptor family [7]. The ligands interacting with ErbB receptors include epigen (EPG), transforming growth factor-α (TGF-α), and Amphiregulin (AR) binding to EGFR; betacellulin (BTC), heparin-binding epidermal growth factor (HB-EGF), and Epiregulin (EPR)

binding to EGFR and ErbB4; neuregulin-1 (Nrg-1) and neuregulin-2 (Nrg-2) binding to ErbB3 and ErbB4; neuregulin-3 (Nrg-3) and neuregulin-4 (Nrg-4) binding to ErbB4 [5]. The EGFR family has been the most investigated receptor protein tyrosine kinase families be-

cause of their role in general signal transduction and in oncogenesis [8]. Out of all the four members of ErbB family, ErbB1 and ErbB2 are the attractive targets for cancers as they are involved in the development and metastases of different cancers [9]. These two pro-



► **Fig. 1** Role of Natural Peptide/TKIs in RAS/mitogen activated protein kinase pathway, PI3K/AKT–mTOR, leading to the inhibition of the important hallmarks of cancer.

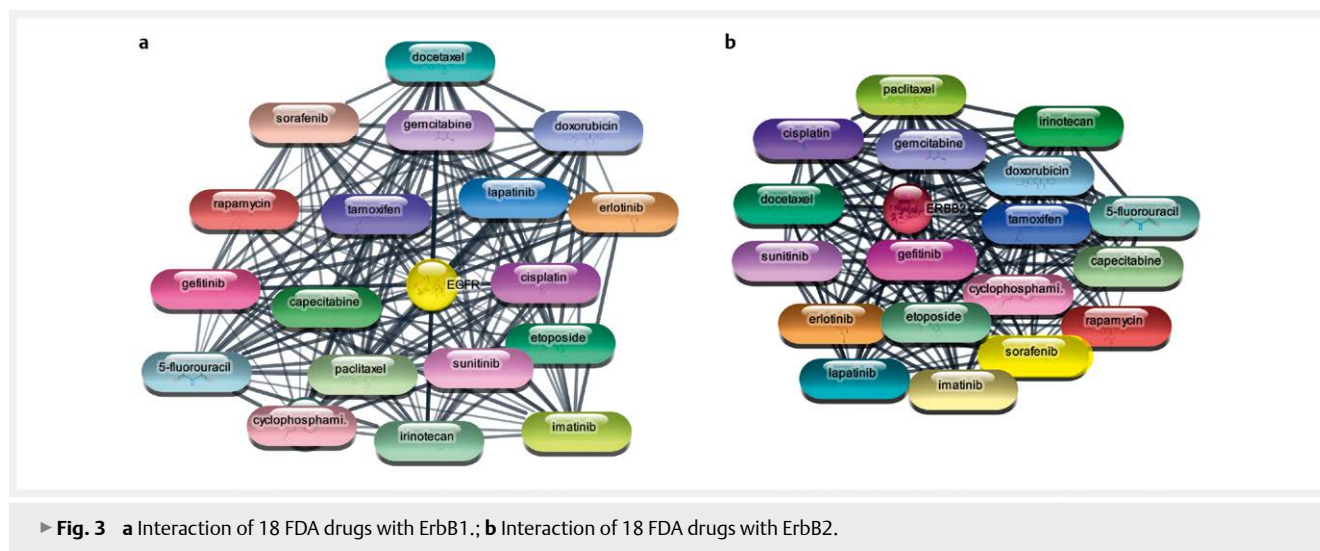


► **Fig. 2** Interaction of 58 FDA drugs with ErbB1.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

teins share high sequence homology and structure, many catalytic and kinetic properties associating distinctly with the cancers [10]. There are many small molecular inhibitors reported as kinase inhibitors. The clinically available first-generation reversible EGFR-TKIs (EGFR-tyrosine kinase inhibitors) such as gefatinib, erlotinib, and icotinib; second generation irreversible EGFR-TKIs such as afatinib, and dacomitinib are being used [11]. Lapatinib and neratinib are clinically used dual inhibitors of ErbB1 and ErbB2. But, cancer cells can develop resistance against these anticancer agents [11, 12]. Hence there is a need to develop alternate drugs which can kill cancer cells effectively without resistance being developed by cancer cells. One such category of drugs includes use of peptides as anticancer drugs. Natural peptides have demonstrated selective cytotoxicity to human cancer cells without effecting the normal

cells [11]. Natural peptides internalize in the intracellular region of EGFRs for the signal attenuation. They bind to the active intracellular kinase domain (ATP binding sites) at the C-terminal and thereby inhibit the phosphorylation at the tyrosine kinase domain which is the preliminary step in the progression of the cancer cells. When the phosphorylation step is inhibited, the adapter proteins do not have phosphorylated residues for further progression in downregulation pathways. This results in the prevention of the phosphotyrosine residues-activating molecules complex formation which further results in the inhibition of RAS/MAPK, PI3K/AKT-mTOR, signal transducers and activators of transcription signaling. This leads to the downregulation of proliferation, invasion, metastases, and angiogenesis of cancer cells (► Fig. 1) [11, 13–15]. Keeping all the facts in our mind, we wanted to identify new ErbB1 and ErbB2 dual



► Table.1 PDI Data using cytoscape against ErbB1.

S. No	Name of the Drug	Degree	Closeness	Betweenness	MNC	Bottleneck	EcCentricity
1.	Tamoxifen	16	17.5	0.11765	16	1	0.5
2.	Doxorubicin	18	18.5	0.36765	18	1	0.5
3.	Cisplatin	18	18.5	0.36765	18	1	0.5
4.	Cyclophosphamide	18	18.5	0.36765	18	1	0.5
5.	Docetaxel	18	18.5	0.36765	18	1	0.5
6.	5-fluorouracil	17	18	0.11765	17	1	0.5
7.	Irinotecan	18	18.5	0.36765	18	1	0.5
8.	Paclitaxel	17	18	0.25	17	1	0.5
9.	Imatinib	1	10	0	1	1	0.5
10.	Gefatinib	18	18.5	0.36765	18	1	0.5
11.	Erlotinib	18	18.5	0.36765	18	1	0.5
12.	Sorafenib	17	18	0.25	17	1	0.5
13.	Sunitinib	18	18.5	0.36765	18	1	0.5
14.	Etoposide	19	19	36.36765	18	2	1
15.	Gemcitabine	17	18	0.11765	17	1	0.5
16.	Capecitabine	18	18.5	0.36765	18	1	0.5
17.	Lapatinib	18	18.5	0.36765	18	1	0.5
18.	Rapamycin	18	18.5	0.36765	18	1	0.5

► **Table.2** PDI Data using cytoscape against ErbB2.

S. No	Name of the Drug	Degree	Closeness	Betweenness	MNC	Bottleneck	EcCentricity
1.	Tamoxifen	18	18	0.36765	18	1	1
2.	Doxorubicin	18	18	0.36765	18	1	1
3.	Cisplatin	18	18	0.36765	18	1	1
4.	Cyclophosphamide	18	18	0.36765	18	1	1
5.	Docetaxel	18	18	0.36765	18	1	1
6.	5-fluorouracil	18	18	0.36765	18	1	1
7.	Irinotecan	18	18	0.36765	18	1	1
8.	Paclitaxel	18	18	0.36765	18	1	1
9.	Imatinib	18	18	0.36765	18	1	1
10.	Gefatinib	18	18	0.36765	18	1	1
11.	Erlotinib	18	18	0.36765	18	1	1
12.	Sorafenib	18	18	0.36765	18	1	1
13.	Sunitinib	18	18	0.36765	18	1	1
14.	Etoposide	17	17.5	0.25	17	1	0.5
15.	Gemcitabine	17	17.5	0.11765	17	1	0.5
16.	Capecitabine	17	17.5	0.11765	17	1	0.5
17.	Lapatinib	17	17.5	0.25	17	1	0.5
18.	Rapamycin	16	17	0.11765	16	1	0.5

► **Table.3** Docking score of FDA approved drugs with ErbB1 and ErbB2 using iGEMDOCK.

Ligand	Binding Energy (kcal/mol)	
	ErbB1	ErbB2
Fluorouracil	-92.46	-97.14
Cyclophosphamide	-83.74	-76.24
Docetaxel	-105.38	-82.59
Doxorubicin	-129.68	-127.28
Erlotinib	-105.96	-120.73
Gefetinib	-106.01	-109.60
Imatinib	-123.87	-118.31
Paclitaxel	-89.63	-100.11
Sorafenib	-118.56	-118.66
Irinotecan	-115.59	-132.97
Sunitinib	-105.49	-108.65
Lapatinib (Standard)	-108.90	-102.70

inhibitors from peptide origin. We also carried out the study with FDA approved drugs and compared the data with peptides.

Materials and methods

Collection and preparation of Proteins ErbB1 and ErbB2

The protein structure of EGFRs, ErbB1 (PDB ID: 1XKK) [16] and ErbB2 (PDB ID: 3PP0) [17], were obtained from the protein data bank (<https://www.rcsb.org>) [18].

Collection of Peptides and FDA approved drugs

A library of natural peptides of five amino acid sequence from NeuroPep database, which is a comprehensive resource of neuropeptides originating from organisms [19] has been retrieved. The list of 2637 FDA approved drugs was obtained from the internet source.

Protein drug interactions network by Cytoscape

The protein drug interaction networks (PDI) was visualized using the Cytoscape 3.9.1 software. Cytoscape 3.9.1 is an open source bioinformatics software used to visualize large sets of biological data and represent them as a network to better understand the relationship between the entities [20]. Our main focus was to establish a network for protein-drug interaction which would provide with a clear picture regarding the selected drugs that can directly interact with both the proteins ErbB1 and ErbB2 [21].

Docking tools

Docking analysis was carried out using iGEMDOCK and online software HPEPDOCK. iGEMDOCK derives the pharmacological interactions of the selected ligands with proteins without the taking the known standard compounds. iGEMDOCK provides a post screening analysis module convenient for the clustering compounds and visualization of pharmacological interactions by interaction profiles [22]. HPEPDOCK is a server for blind peptide-protein docking by fast modeling of peptide conformations and global sampling of binding orientations [23]. FDA drugs were docked using iGEMDOCK, whereas, for peptides double docking was performed using iGEMDOCK and HPEPDOCK.

ADMET analysis

ADMET analysis was performed using DataWarrior software, which is an open-source program for the data visualization and analysis

with chemical intelligence. DataWarrior combines dynamic graphical views and interactive row filtering with chemical intelligence [24].

Invitro Proliferation assay (MTT assay)

Based on the docking results top peptide (YSFGL) and top FDA approved drug (Doxorubicin) have been selected for biological activity by MTT assay and compared with the standard lapatinib.

The obtained human lung cancer and breast cancer cell lines (A549 and MDA-MB-231) were cultured in DMEM medium supplemented with 10 percent fetal bovine serum (FBS) and 1 percent penicillin streptomycin at 37 °C in a humidified 5 percent CO₂ atmosphere. Cells were cultured (1 × 10⁴ cells/well) into flat-bottom 96-well plates (Corning[®] Cell Bind[®] Surface), in triplicate amount of 100 µL of cell suspension with media per each well. In order to determine the cytotoxicity effect of the peptide YSFGL, doxorubicin and standard lapatinib, the cell lines were treated with the compounds, which were freshly dissolved in the cell culture medium, at different concentrations (100, 50, 25, 12.50 and 6.25 µM) for 48 h. The cells of the control group and blank group were left untreated. After 48 h of treatment, MTT reagent at a final concentration of 0.5 mg/ml was added to each well, and incubated for 4 h at 37 °C. The blue-colored product was solubilized in DMSO and finally absorbance was measured at 570 nm using Varioskan[™] Flash Multimode Reader (Thermo Scientific) [25]. Data obtained are expressed as the percentage of control mean ± SD of triplicate values. The IC₅₀ was determined by using GraphPad Prism software version 9.0.0 and nonlinear regression (curve fit).

Results

Protein drug interactions network by Cytoscape

All the 2637 FDA approved drugs were loaded to the cytoscape software and protein-drug interactions were recorded against ErbB1 and 58 drugs showed interaction with ErbB1 (► Fig. 2). These

drugs were studied for the interactions with ErbB2 and 18 drugs showed interaction (► Fig. 3b). These 18 drugs can be the dual inhibitors of ErbB1 (► Table. 1) and ErbB2 (► Table.2) (► Fig. 3a, b). The final drugs were then docked against ErbB1 and ErbB2 using iGEMDOCK and the results were compared with the docking scores of peptides.

Molecular docking

All the FDA approved drugs and Neuropep peptides were subjected to the molecular docking using iGEMDOCK. The FDA approved drugs showed binding energy (► Table.3) ranging from -83.74 kcal/mol to -129.68 kcal/mol for ErbB1 and -76.24 kcal/mol to -132.97 kcal/mol for ErbB2. The average binding energy showed by doxorubicin is higher than all the other FDA approved drugs with -129.68 kcal/mol for ErbB1 and -127.28 kcal/mol for ErbB2. From the binding energies it may be noted that this drug can act as a dual inhibitor of ErbB1 and ErbB2. Double docking was done for the peptides using HPEPDOCK and iGEMDOCK (► Table.4). The average binding energy showed by YSFGL is higher than all the other peptides with -116.23 kcal/mol for ErbB1 and -107.06 kcal/mol for ErbB2 using HPEPDOCK; -114.63 kcal/mol for ErbB1 and -120.62 kcal/mol for ErbB2 using iGEMDOCK.

ADMET analysis

The ADMET analysis by using DataWarrior for FDA approved drugs and Neuropep peptides are showed in ► Table.5 and ► Table.6. The molecular weight of top 11 FDA drugs were in the range of 246.193 kDa to 853.915 kDa and molecular weight of top 11 peptides were in the range of 429.475 kDa to 683.828 kDa. Cyclophosphamide showed mutagenicity, tumorigenicity and effect on reproductive system, Doxorubicin and Sunitinib showed irritation whereas, peptides did not show any effect.

Invitro Proliferation (MTT) Assay

After treatment against the cell lines, the cytotoxic activity of the test compounds was illustrated in ► Table.7, ► Fig. 4, 5. The doxo-

► Table.4 Docking score of Neuropep peptides with ErbB1 and ErbB2.

Ligands	Binding Energy (kcal/mol)			
	HPEPDOCK		iGEMDOCK	
	ErbB1	ErbB2	ErbB1	ErbB2
YAFGL	-103.24	-100.28	-119.86	-116.44
YSFGL	-116.23	-107.06	-114.63	-120.62
TLFRF	-108.4	-104.82	-105.66	-118.59
YLRF	-94.098	-138.65	-105.8	-105.99
YPFF	-99.352	-110.88	-101.77	-100.08
YGFL	-99.985	-105.91	-109.92	-118.32
YPWG	-97.51	-102.85	-101.09	-101.35
YPWT	-115.21	-100.97	-102.37	-96.439
FYRI	-110.24	-98.285	-113.96	-105.61
FLRN	-100.24	-86.233	-105.18	-104.68
APGW	-113.88	-69.344	-109.89	-103.26
Lapatinib (Standard)	-	-	-108.90	-102.70
(No peptide standards are available for the selected targets)				

▶ **Table.5** ADMET analysis data of FDA approved drugs.

Drug name	Total Mol. weight	cLogP	clogS	H-Acceptors	H-Donors	Polar Surface Area	Drug likeness	Muta-genic	Tumori-genic	Reproductive Effective	Irritant	Rotatable Bonds
Flurouracil	246.193	-1.4898	-1.503	7	3	99.1	-3.4013	None	None	None	None	2
Cyclophosphamide	263.104	-0.0289	-2.823	4	2	44.73	-9.8384	High	High	High	None	5
Docetaxel	807.887	2.609	-5.811	15	5	224.45	-55.654	None	None	None	None	13
Doxorubicin	543.523	0.1673	-4.507	12	6	206.07	6.6484	None	None	None	High	5
Erlotinib	393.442	3.0713	-3.527	7	1	74.73	-5.9718	None	None	None	None	10
Gefetinib	446.909	3.9851	-5.062	7	1	68.74	0.47937	None	None	None	None	8
Imatinib	493.613	3.9383	-4.383	8	2	86.28	8.6128	None	None	None	None	7
Paclitaxel	853.915	3.188	-6.289	15	4	221.29	0.82049	None	None	None	None	14
Sorafenib	464.83	4.1428	-6.689	7	3	92.35	-5.1185	None	None	None	None	6
Irinotecan	586.687	3.5596	-4.504	10	1	112.51	-0.08661	None	None	None	None	5
Sunitinib	398.48	1.836	-3.471	6	3	77.23	8.335	None	None	None	High	7

▶ **Table.6** ADMET analysis data of Neuropeptide peptides.

Peptide sequence	Total Molweight	cLogP	cLogS	H-Acceptors	H-Donors	Polar Surface Area	Druglike-ness	Muta-genic	Tumori-genic	Reproductive Effective	Irritant	Rotatable Bonds
YGFL	498.578	-1.3562	-3.137	10	6	170.85	-12.376	None	None	None	None	13
YLRF	598.722	-2.0759	-4.225	13	9	236.86	-11.858	None	None	None	None	17
YPFF	572.66	-0.0655	-3.974	10	5	162.06	-8.4144	None	None	None	None	12
YPWG	521.572	-1.8273	-2.986	11	6	177.85	-1.3137	None	None	None	None	10
YPWT	565.625	-2.0354	-3.235	12	7	198.08	-5.3323	None	None	None	None	11
YSFGL	585.656	-2.8399	-2.775	13	8	220.18	-11.457	None	None	None	None	16
FYRI	598.722	-2.0759	-4.225	13	9	236.86	-7.5668	None	None	None	None	17
TLFRF	683.828	-2.8546	-4.537	15	10	265.96	-14.789	None	None	None	None	20
YAFGL	569.657	-1.9132	-3.282	12	7	199.95	-12.516	None	None	None	None	15
APGW	429.475	-2.9235	-2.147	10	5	157.62	-4.802	None	None	None	None	8
FLRN	549.651	-4.3918	-3.207	14	9	259.72	-12.71	None	None	None	None	17

(Limits - Mol. Wt:<500 Daltons [for small molecules]; clogP:<5; clogS:>-5; H-acceptors:<10; H-Donors:<5; Polar Surface area:<200; Mutagenicity: None; Tumorigenicity: None; Reproductive Effect: None; Irritation: None; Rotatable Bonds:<5).

rubicin showed significant cytotoxic effect against A549 cells with IC₅₀ value of 7.675 ± 0.278 μM and YSFGL showed cytotoxicity with IC₅₀ value of 26.417 ± 0.660 μM, in comparison with the standard lapatinib (2.380 ± 0.357 μM). The IC₅₀ value of doxorubicin and YSFGL were found to be 8.313 ± 0.435 μM and 39.047 ± 0.770 μM respectively against MDA-MB-231 cell lines as compared with the standard lapatinib (3.026 ± 0.180 μM).

Conclusion

Several biopharmaceutical agents have been approved by FDA for the treatment of cancer. A major shortcoming of these drugs is the development of resistance. So, there is an immediate need for the discovery of the alternate agents for treatment of cancer without developing resistance by cancer cells. In the present study, we focused mainly on the discovery of potent inhibitors of ErbB1 and ErbB2 as a potential therapy for treatment of cancer using *insilico* methods. The already existing FDA approved drugs against various

diseases have been studied for interactions against ErbB1 and ErbB2 using cytoscape software. The results suggested that the drugs have interacted against the both proteins, and can be repurposed as anticancer agents. Then, molecular docking studies have been conducted to explore the possible binding of the FDA approved drugs and Neuropep peptides against ErbB1 and ErbB2 for their anticancer activity. Our binding results suggested the possible binding and the role of the selected drugs as dual inhibitors, thereby, leading for the development of these drugs as new entities for anticancer activity. The ADMET analysis of other drugs and peptides suggested that these drugs follows the limits of ADMET properties and can be established as therapeutic agents. The *invitro* MTT assay suggested that doxorubicin and peptide YSFGL showed significant anticancer activity against A549 and MDA-MB-231 cell lines. Further studies on these agents can be performed for exploring their role in treatment of cancer where ErbB1 and ErbB2 play a key role in progression of cancer.

► **Table.7** IC₅₀ values of test drugs against cell lines.

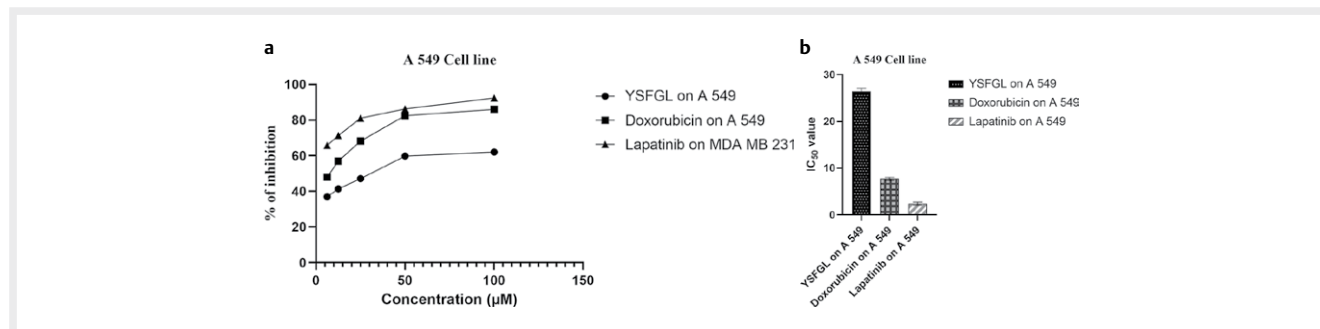
Drug name	IC ₅₀ Values (μM)	
	A549	MDA-MB-231
YSFGL	26.417 ± 0.660	39.047 ± 0.770
Doxorubicin	7.675 ± 0.278	8.313 ± 0.435
Lapatinib (standard)	2.380 ± 0.357	3.026 ± 0.180

Data availability statement

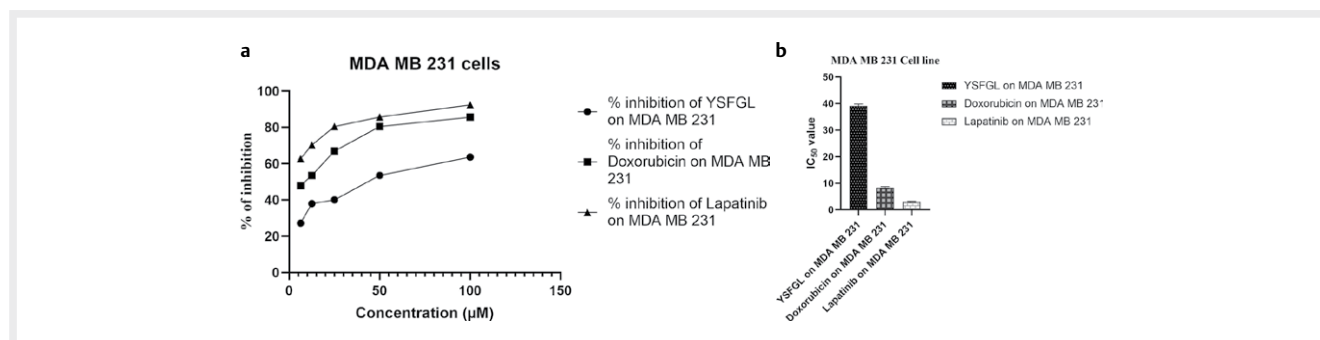
All data generated and analyzed during this study are included within this article

Ethical Approval

Not Required



► **Fig. 4** a Plot showing % inhibition of A 549.; b Plot showing IC50 value on A 549.



► **Fig.5** a Plot showing % inhibition of MDA-MB-231; b. Plot showing IC50 value on MDA-MB-231

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, data curation: **Sunil Kumar Patnaik, Akey Krishna Swaroop, Mudavath Ravi Naik**; writing-original draft preparation: **Sunil Kumar Patnaik**, review, editing and supervision: **Dr. Moola Joghee Nanjan Chandrasekar, Dr. Jubie Selvaraj**

Acknowledgments

Authors express their immense gratitude to JSS College of Pharmacy Ooty, JSS Academy of Higher Education and Research, Mysuru, and School of Life Sciences, JSS Academy of Higher Education & Research (Ooty Campus), Longwood, Mysuru Road, Ooty-643001, Tamilnadu, India for the facilities offered.

Funding

No funding was provided for the above mentioned research

Conflict of Interest

The authors declare that they have no conflict of interests

References

- [1] Iheagwam FN, Ogunlana OO, Ogunlana OE et al. Potential anti-cancer flavonoids isolated from *Caesalpinia bonduca* young twigs and leaves: molecular docking and in silico studies. *Bioinformatics and Biology Insights* 2019; 13: 1177932218821371
- [2] Chakravarti B, Maurya R, Siddiqui JA et al. In vitro anti-breast cancer activity of ethanolic extract of *Wrightia tomentosa*: role of proapoptotic effects of oleanolic acid and urosolic acid. *Journal of Ethnopharmacology* 2012; 142: 72–79
- [3] Kakde D, Jain D, Shrivastava V et al. Cancer therapeutics-opportunities, challenges and advances in drug delivery. *Journal of Applied Pharmaceutical Science* 2011; 01–10
- [4] Roskoski R Jr. The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacological Research* 2014; 79: 34–74
- [5] Roskoski R Jr. ErbB/HER protein-tyrosine kinases: Structures and small molecule inhibitors. *Pharmacological Research* 2014; 87: 42–59
- [6] Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010; 141: 1117–1134
- [7] Ahsan A, Ramanand SG, Bergin IL et al. Efficacy of an EGFR-specific peptide against EGFR-dependent cancer cell lines and tumor xenografts. *Neoplasia* 2014; 16: 105–W2
- [8] Roskoski R Jr. Small molecule inhibitors targeting the EGFR/ErbB family of protein-tyrosine kinases in human cancers. *Pharmacological Research* 2019; 139: 395–411
- [9] Normanno N, De Luca A, Bianco C et al. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* 2006; 366: 2–16
- [10] Deng Y, Li J. Rational Optimization of Tumor Suppressor-Derived Peptide Inhibitor Selectivity between Oncogene Tyrosine Kinases ErbB1 and ErbB2. *Archiv Der Pharmazie* 2017; 350: 1700181
- [11] Patnaik SK, Chandrasekar MJN, Nagarjuna P et al. Targeting of ErbB1, ErbB2, and their Dual Targeting Using Small Molecules and Natural Peptides: Blocking EGFR Cell Signaling Pathways in Cancer: A Mini Review. *Mini Reviews in Medicinal Chemistry* 2022
- [12] Zhong L, Li Y, Xiong L et al. Small molecules in targeted cancer therapy: Advances, challenges, and future perspectives. *Signal Transduction and Targeted Therapy* 2021; 6: 1–48.
- [13] Wee P, Wang Z. Epidermal growth factor receptor cell proliferation signaling pathways. *Cancers* 2017; 9: 52
- [14] Spector NL, Xia W, Burris H III et al. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *Journal of Clinical Oncology* 2005; 23: 2502–2512
- [15] Mine Y, Munir H, Nakanishi Y et al. Biomimetic peptides for the treatment of cancer. *Anticancer Research* 2016; 36: 3565–3570
- [16] Wood ER, Truesdale AT, McDonald OB et al. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res* 2004; 64: 6652–6659. doi:10.1158/0008-5472.CAN-04-1168
- [17] Aertgeerts K, Skene R, Yano J et al. Structural analysis of the mechanism of inhibition and allosteric activation of the kinase domain of HER2 protein. *Journal of Biological Chemistry* 2011; 286: 18756–18765
- [18] Berman HM, Westbrook J, Feng Z et al. The protein data Bank nucleic acids research. 2000. Europe PMC Free Article][Abstract][Google Scholar] 2021; 235–242
- [19] Wang Y, Wang M, Yin S et al. NeuroPep: a comprehensive resource of neuropeptides. *Database* 2015. 2015: doi:10.1093/database/bav038
- [20] Killcoyne S, Carter GW, Smith J et al. Cytoscape: a community-based framework for network modeling. *Methods Mol Biol* 2009; 563: 219–239. doi:10.1007/978-1-60761-175-2_12
- [21] Chen S. Network analysis of Urocortins. *Neuroendocrinology Letters* 2016; 37: 461–466
- [22] Hsu K-C, Chen Y-F, Lin S-R et al. iGEMDOCK: a graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. *BMC Bioinformatics* 2011; 12: 1–11.
- [23] Zhou P, Jin B, Li H et al. HPEPDOCK: a web server for blind peptide–protein docking based on a hierarchical algorithm. *Nucleic Acids Research* 2018; 46: W443–W450
- [24] Sander T, Freyss J, von Korff M et al. DataWarrior: an open-source program for chemistry aware data visualization and analysis. *Journal of Chemical Information and Modeling* 2015; 55: 460–473
- [25] Meerloo JV, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. *Cancer cell culture*. Springer; 2011: 237–245